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Laustsen, Andreas H.

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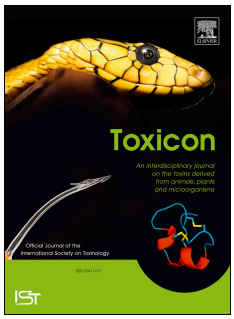

Toxin-centric development approach for next-generation antivenoms

Andreas H. Laustsen

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Correspondence

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With its recent re-introduction on the World Health Organization's list of Neglected Tropical Diseases (Chippaux, 2017), snakebite envenoming is gaining increased international attention. Not only does this create hope that new policies and guidelines may help healthcare systems to implement better preventive strategies, but it may also create incentive for research and development efforts aiming at delivering new antivenom products for treatment of snakebite envenoming. New products may fall into two categories: 1) Plasma-derived antivenoms based on conventional immunization approaches (Gutiérrez et al., 2011; World Health Organisation, 2010) and 2) next-generation antivenoms based on specific therapeutic molecules that are selected on their ability to neutralize key toxins (Knudsen and Laustsen, 2018), and which are manufactured without the need to procure snake venoms. The benefit of the first type of antivenom products is that their therapeutic benefits have been historically documented (Gutiérrez et al., 2011). However, they suffer from the drawback of not being compatible with the human immune system due to their heterologous nature (Laustsen et al., 2016a), and from having a low content of therapeutically active antibodies, particularly against small venom components with low immunogenicity and high toxicity (Laustsen et al., 2017; Leong et al., 2015; Tan et al., 2015, 2016). In recent time, venomics and antivenomics approaches have helped build a greater understanding of the antigenicity of different venom components, which may provide guidance on how to design immunization mixtures that give rise to venom-paraspecific antibody responses in production animals (Calvete et al., 2014, 2009; Lomonte and Calvete, 2017). Such knowledge creates a foundation for improving the neutralization potential of antivenoms against toxin subfamilies with low immunogenicity, such

as small neurotoxins from the three-finger toxin family (Calvete et al., 2018; Tan et al., 2017). In contrast to plasma-derived antivenoms, the development of next-generation antivenoms is unaffected by any discrepancy between toxicity and immunogenicity, as monoclonal antibodies and small molecule inhibitors can be discovered even for non-immunogenic toxins.

Independent on the particular molecular scaffold (small molecule, antibody, antibody fragment etc.) employed in a next-generation antivenom, a fundamental change in our understanding of snake venoms as drug targets will have to occur. Conventional antivenom manufacture involves the use of whole venoms during the immunization process. For this approach, it is important to optimize the immunization mixture to ensure that the venoms employed are representative of the venoms in the geographic region the antivenom is to be deployed (Gutiérrez, 2007). This necessitates the collection of snakes with venom compositions that are representative of the given region, as such venom compositions have been shown to vary quite dramatically across geographic ranges (Chippaux et al., 1991). Among other things, this possibly creates an incentive for establishing serpentaria in different regions of the world.

In contrast to conventional antivenom manufacture, the manufacture of next-generation antivenoms installs a very different demand, as these antivenoms are not dependent on snake venoms for their manufacture (Laustsen et al., 2016b). A thorough understanding of venom compositions in relation to geographic region is still essential to guide the compositional formulation of next-generation antivenoms (Laustsen, In press), but the need to procure snake venoms in large quantities becomes irrelevant. In fact, the development of specific molecular antitoxins is not even reliant on ‘representative’ whole venoms, if the key toxins can be isolated from a given venom in sufficient quantities for antitoxin discovery purposes (Figure 1).

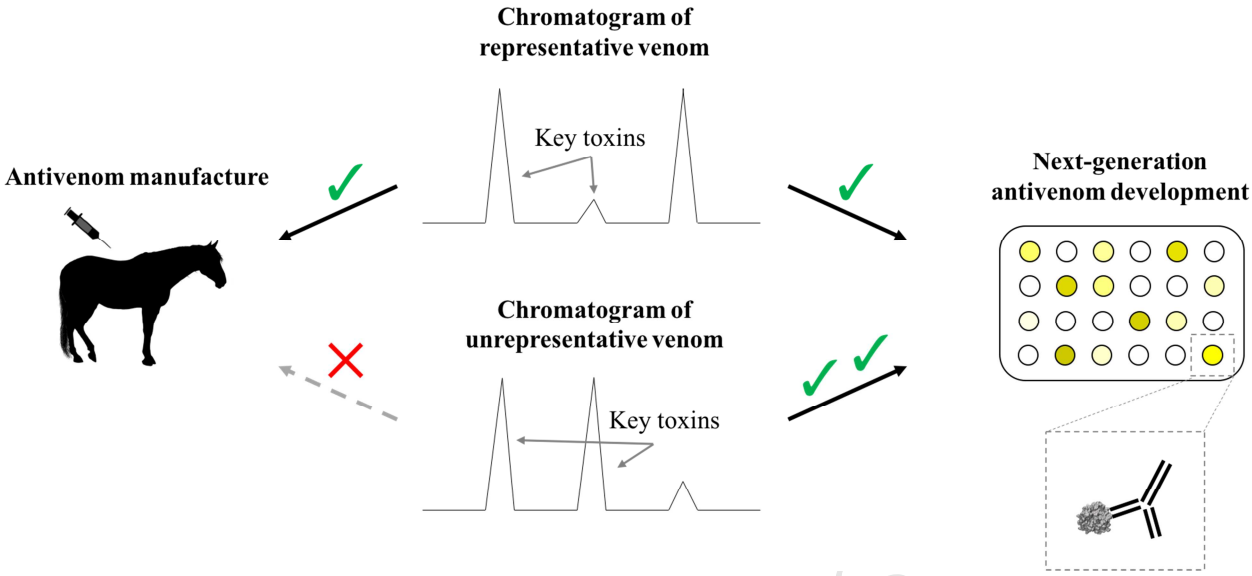

Figure 1. *Schematic representation of chromatograms for two different venoms from the same snake species (one venom that is representative of the venom compositions of snakes in a given geographic region and one venom that is not). For antivenom manufacture, it is important that the venoms employed in the immunization mixture are representative of the venoms in the geographic region that the antivenom is to be deployed. In comparison, a venom does not need to be representative when used for next-generation antivenom development purposes, as the key toxins typically are to be isolated and used in pure form. An unrepresentative venom with high proportions of key toxins will even be more useful than a representative venom with lower proportions.*

The exact timing of when next-generation antivenoms will be introduced in the clinic is unknown. However, the transition from plasma-derived antivenoms to antivenoms based on defined mixtures of synthetic and/or recombinant components seems unlikely not to happen in the future (Laustsen et al., 2016a). With this transition, the need remains for studying snake venom compositions and identifying all toxins that are medically relevant to neutralize (Lomonte and Calvete, 2017), but the focus in antivenom development will indeed change from obtainment of

whole venoms that are representative of certain habitats, age groups, and geographical regions to a more toxin-centric focus, where it is more important to obtain key snake toxins in isolated form than it is to procure representative venoms. With the toxin-centric focus in antivenom development, antitoxin discovery efforts should not aim to identify molecular antitoxins that can target a group of toxins within only one whole venom, but rather focus on neutralization of toxin subfamilies across multiple species and identification of which (conserved) toxin epitopes should be targeted (Engmark et al., 2016, 2017b; Laustsen, In press). This will allow for a mix-and-match approach, where polyvalent next-generation antivenoms can be built up by mixing individual toxin subfamily-specific antitoxins that combined may neutralize all the key toxin subfamilies in several target venoms (Figure 2). It may therefore become more relevant to identify key toxins from different toxin subfamilies that are essential to be neutralized than to identify whole venoms that are representative for a given species (in a given geographic region). In this context, it may also be relevant to explore how broad target specificity can be obtained for different antitoxins, as it will likely be difficult to identify single antitoxins that can cross-neutralize an entire subfamily of toxins (Engmark et al., 2017a), although promising results have been reported for certain inhibitors against enzymatic toxins (Arias et al., 2017; Knudsen and Laustsen, 2018; Lewin et al., 2016).

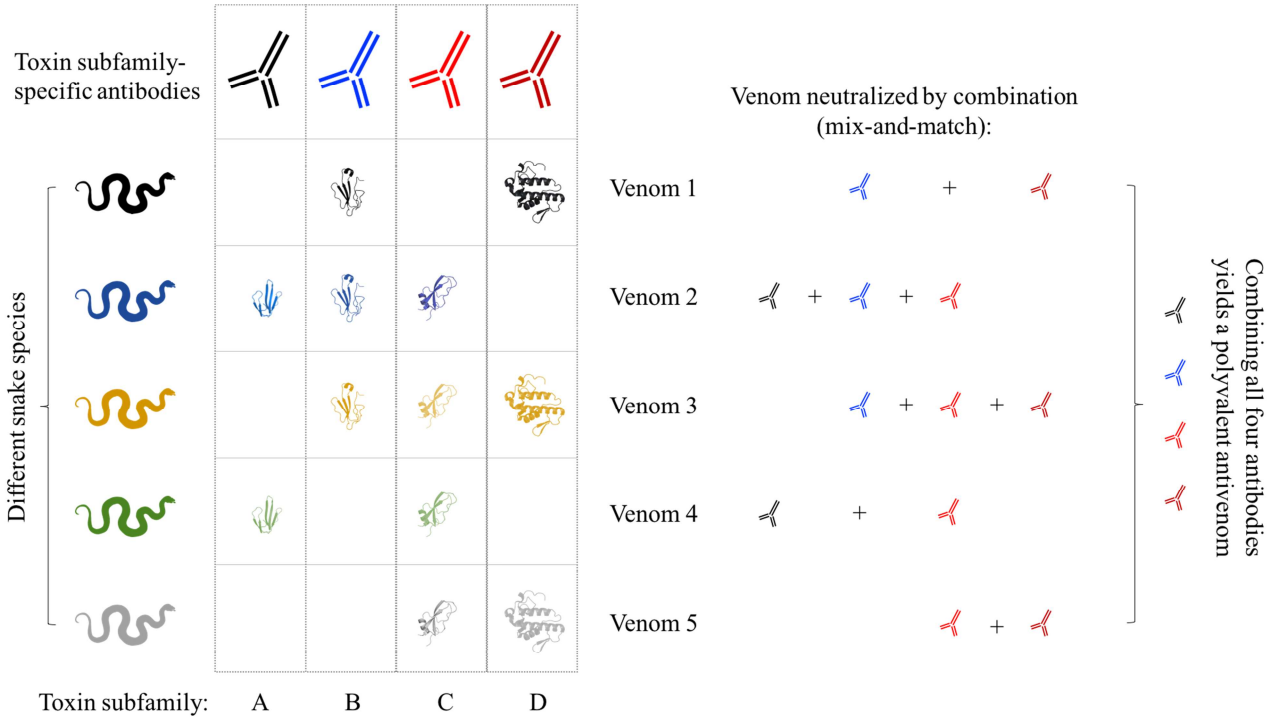

Figure 2: *Schematic overview of how polyvalent next-generation antivenoms can be designed by combining antitoxins (illustrated with IgG antibodies) that can cross-neutralize toxins from the same subfamily across different snake species. This approach requires that focus in antivenom development is shifted from representative whole venoms from specific snake species to becoming more toxin-centric.*

If these predictions hold true, the implications may firstly become significant for governments and healthcare systems that are considering the establishment of new serpentaria to obtain collections of snake specimens with venom compositions that are representative for their geographical region, as such serpentaria risk becoming redundant. Secondly, antivenom researchers may have to re-evaluate how they understand the interplay between toxins and venoms and switch from a currently strong focus on neutralizing whole venoms towards a focus on neutralization of toxin subfamilies across multiple snake species.

Andreas H. Laustsen

Department of Biotechnology and Biomedicine

Technical University of Denmark

Kongens Lyngby, Denmark

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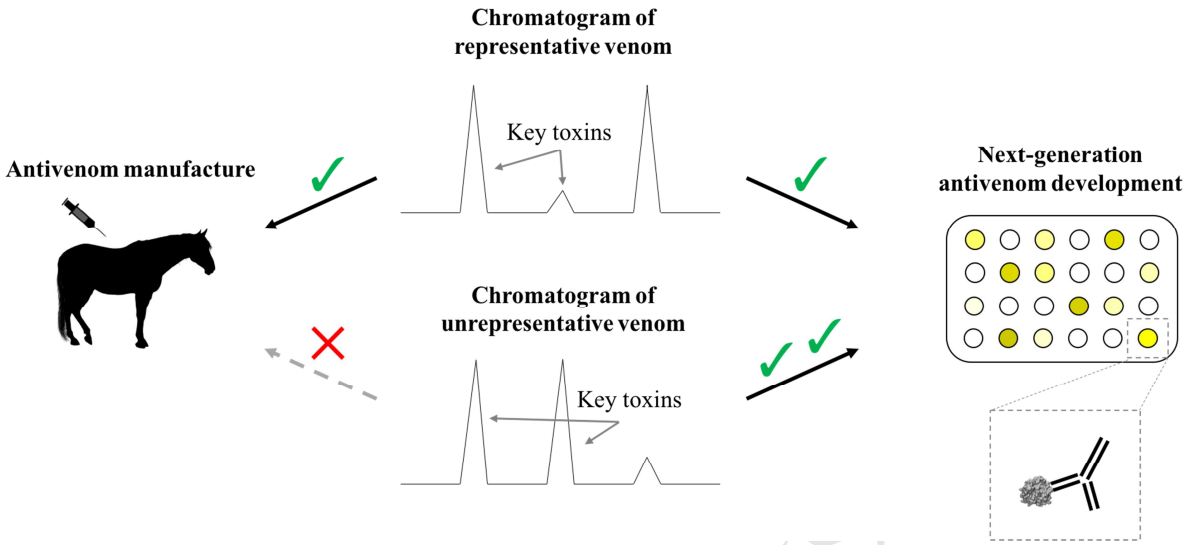

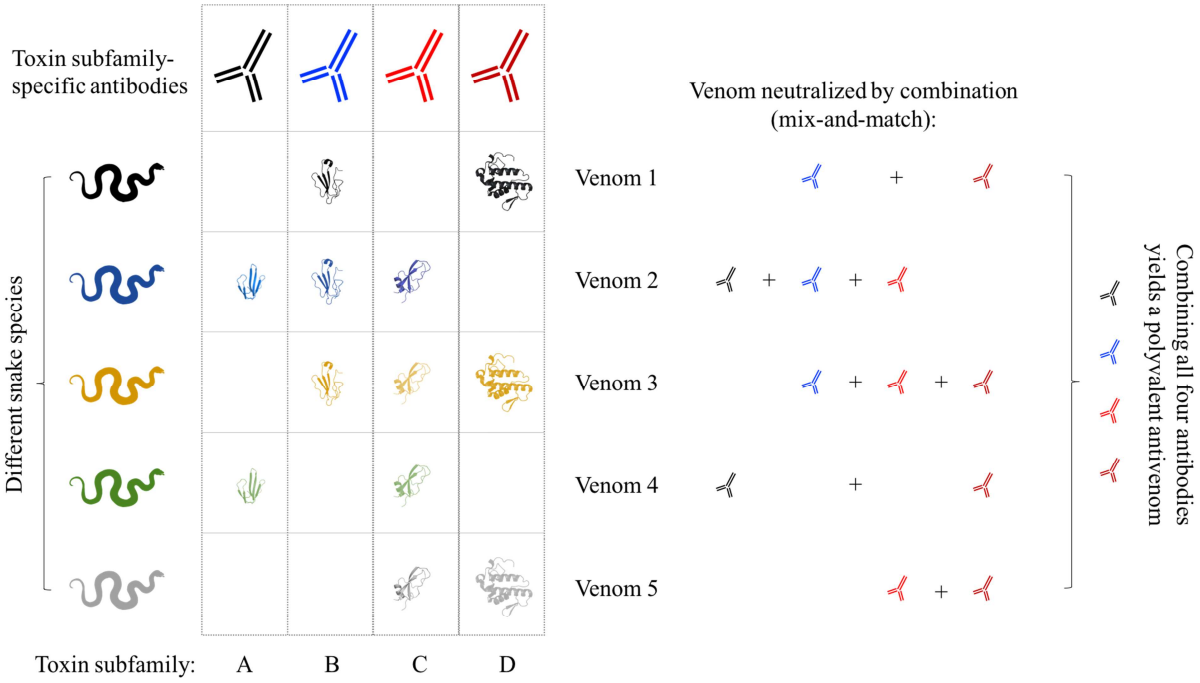

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